09/542,121

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     (FILE 'HOME' ENTERED AT 12:29:32 ON 13 MAR 2002)

     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 12:29:46 ON
     13 MAR 2002
               SEA (LACTIC(W)ACID(W)BACTERIA)
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                 5   FILE ADISALERTS
                 1   FILE ADISINSIGHT
                 2*  FILE ADISNEWS
              2510   FILE AGRICOLA
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               106   FILE AQUASCI
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                76   FILE BIOCOMMERCE
              3974   FILE BIOSIS
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              5952   FILE CABA
                62   FILE CANCERLIT
              6663   FILE CAPLUS
               446   FILE CEABA-VTB
                 1   FILE CEN
                23   FILE CIN
                82   FILE CONFSCI
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               466   FILE GENBANK
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              1994   FILE LIFESCI
                11   FILE MEDICONF
              1303   FILE MEDLINE
                 1   FILE NIOSHTIC
                31   FILE NTIS
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              4532   FILE PASCAL
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                1   FILE PHAR
                1   FILE [redacted]HIC
               23   FILE [redacted]HIN
              325   FILE PROMT
             4431   FILE SCISEARCH
             1222   FILE TOXCENTER
              547   FILE TOXLIT
              910   FILE USPATFULL
                1   FILE USPAT2
             1528   FILE WPIDS
             1528   FILE WPINDEX
L1               QUE (LACTIC(W) ACID(W) BACTERIA)
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     FILE 'CAPLUS, CABA, FROSTI, FSTA, PASCAL, BIOSIS, SCISEARCH' ENTERED AT
     12:32:13 ON 13 MAR 2002
L2           441 S L1 AND (BETA-GALACTOSIDASE)
L3             0 S L2 AND (4000(W)MILLER UNITS)
L4             0 S L2 AND (MILLER UNITS)
L5             0 S L2 AND MILLER
L6         59670 S BETA-GALACTOSIDASE
L7            53 S L6 AND (MILLER(W)UNITS)
L8             0 S L7 AND (4000(W)MILLER)
L9            19 DUP REM L7 (34 DUPLICATES REMOVED)
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=> d l9 ibib ab 1-19

L9 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:15400 CAPLUS
TITLE: Measuring .**beta**.-**Galactosidase** Activity in Bacteria: Cell Growth, Permeabilization, and Enzyme Assays in 96-Well Arrays
AUTHOR(S): Griffith, Kevin L.; Wolf, Richard E., Jr.
CORPORATE SOURCE: Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, 21250, USA
SOURCE: Biochemical and Biophysical Research Communications (2002), 290(1), 397-402
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We describe a high-throughput procedure for measuring .**beta**.-**galactosidase** activity in bacteria. This procedure is unique because all manipulations, including bacterial growth and cell permeabilization, are performed in a 96-well format. Cells are permeabilized by chloroform/SDS treatment directly in the 96-well blocks and then transferred to 96-well microplates for std. colorimetric assay of .**beta**.-**galactosidase** activity as described by Miller [J. H. Miller (1972) Expts. in Mol. Genetics, Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY]. Absorbance data are collected with a microplate reader and analyzed using a Microsoft Excel spreadsheet. The .**beta**.-**galactosidase** specific activity values obtained with the high-throughput procedure are identical to those obtained by the traditional single-tube method of Miller. Thus, values obtained with this procedure may be expressed as **Miller units** and compared directly to **Miller units** reported in the literature. The 96-well format for permeabilization and assay of enzyme specific activity together with the use of 12-channel and repeater pipettors enables efficient processing of hundreds of samples in an 8-h day. (c) 2002 Academic Press.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:170031 CAPLUS
DOCUMENT NUMBER: 135:267885
TITLE: pAM401-based shuttle vectors that enable overexpression of promoterless genes and one-step purification of tag fusion proteins directly from Enterococcus faecalis
AUTHOR(S): Fujimoto, Shuhei; Ike, Yasuyoshi
CORPORATE SOURCE: Department of Microbiology School of Medicine, Gunma University, Gunma, 371-8511, Japan
SOURCE: Appl. Environ. Microbiol. (2001), 67(3), 1262-1267
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two novel Enterococcus faecalis-Escherichia coli shuttle vectors that

utilize the promoter and ribosome binding site of bacA on the E. faecalis plasmid pPD1 were constructed. The vectors were named pMGS100 and pMGS101. PMGS100 was designed to overexpress cloned genes in E. coli and E. faecalis and encodes the bacA promoter followed by a cloning site and stop codon. PMGS101 was designed for the overexpression and purifn. of a cloned protein fused to a Strep-tag consisting of 9 amino acids at the carboxyl terminus. The Strep-tag provides the cloned protein with an affinity to immobilized streptavidin that facilitates protein purifn. The authors cloned a promoterless .**beta**.-**galactosidase** gene from E. coli and cloned the traA gene of the E. faecalis plasmid pAD1 into the vectors to test gene expression and protein purifn., resp. .**beta**.-**Galactosidase** was expressed in E. coli and E. faecalis at levels of 103 and 10 **Miller units**, resp. By cloning the pAD1 traA into pMGS101, the protein could be purified directly from a crude lysate of E. faecalis or E. coli with an immobilized streptavidin matrix by one-step affinity chromatog. The ability of TraA to bind DNA was demonstrated by the DNA-assocd. protein tag affinity chromatog. method using lysates prepd. from both E. coli and E. faecalis that overexpress TraA. The results demonstrated the usefulness of the vectors for the overexpression and cis/trans anal. of regulatory genes, purifn. and copurifn. of proteins from E. faecalis, DNA binding anal., detn. of translation initiation site, and other applications that require proteins purified from E. faecalis.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3

ACCESSION NUMBER: 2001:167568 CAPLUS
DOCUMENT NUMBER: 134:339564
TITLE: Characterization of an oxygen-dependent inducible promoter, the nar promoter of Escherichia coli, to utilize in metabolic engineering
AUTHOR(S): Han, Se Jong; Chang, Ho Nam; Lee, Jongwon
CORPORATE SOURCE: Department of Chemical Engineering and Bioprocess Engineering Research Center, Taejon, S. Korea
SOURCE: Biotechnology and Bioengineering (2001), 72(5), 573-576
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nar promoters, whose transcription is maximally induced under microaerobic conditions in the presence of nitrate ion, were characterized in fed-batch culture to det. whether they can be used for metabolic engineering, by which overall prodn. of valuable chems. can be increased. For this purpose, we tested whether the expression level of a reporter gene, the lacZ gene from the nar promoter, could be maintained const. throughout the induction period by manipulation of dissolved oxygen (DO) levels at a given nitrate ion concn. First, E. coli was grown under aerobic conditions (DO 80%) to absorbance at 600 nm (OD600) of 35, then the nar promoter was induced by redn. of DO to different levels, combined with different frequencies and duration of alternating microaerobic and aerobic conditions throughout the entire induction period. For a wild-type nar promoter (pMW61) in a mutant host E. coli with a mutation in the narG gene on the chromosome of the host (RK5265), it was possible to maintain prodn. of .**beta**.-**galactosidase** activity per cell (specific .**beta**.-**galactosidase** activity) at a const. rate at 5000, 10,000, 15,000, and 20,000 **Miller units**, using different combinations of nitrate ion concns. (0.1%,

0.5%, and 1%) and DO levels. In addn., it was possible to maintain prodn.
of specific .**beta.-galactosidase** activity at a cons[illegible]
rate at about 10,000 **Miller units** in the absence of
nitrate ion when a nitrate-independent nar promoter (pMW618) in the narL-
mutant of the W3110 E. coli strain (W3110narL-) was used. Based on these
results, we conclude that the nar promoter system provides a convenient
expression system for metabolic engineering as well as for maximal prodn.
of recombinant proteins under conditions of fed-batch culture.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 2001:6607 CAPLUS
DOCUMENT NUMBER: 134:126627
TITLE: Coupling the T7 A1 promoter to the runaway-replication
vector as an efficient method for stringent control
and high-level expression of lacZ
AUTHOR(S): Chao, Yun-Peng; Chern, Jong-Tzer; Wen, Chih-Sheng
CORPORATE SOURCE: Department of Chemical Engineering, Feng Chia University, Taichung, Taiwan
SOURCE: Biotechnology Progress (2001), 17(1), 203-207
CODEN: BIPRET; ISSN: 8756-7938
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An expression vector characterized by tight regulation and high expression
of cloned genes appears to be indispensable for the engineering need. To
achieve this goal, in assocn. with lacI the T7 A1 promoter contg. two
synthetic lac operators was constructed into a runaway-replication vector.
To further examine this vector system, lacZ was subcloned and placed under
the control of the T7 A1 promoter on the plasmid. With the application of
the thermal induction alone, the Escherichia coli strain harboring the
recombinant plasmid was able to produce 15,000 **Miller units** of .**beta.-galactosidase**, while it
yielded the recombinant protein with 45,000-50,000 **Miller units** upon both thermal and chem. induction. In sharp contrast,
only 60-90 **Miller units** of .**beta.-galactosidase** was obtained for the cell at an uninduced state. As
a result, the prodn. yield of .**beta.-galactosidase** over the background level is amplified approx. 170-fold by thermal
induction and 500-fold by thermal and chem. induction. To produce the
recombinant protein on a large scale, an approach by connecting two
fermenters in series was newly developed. By applying the three-stage
temp. shift in this dual fermenter system, 55,000 **Miller units** of .**beta.-galactosidase** was obtained.
Overall, it shows the potential use of the vector system developed here
for its tight control and high prodn. of recombinant proteins.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 5 OF 19 FSTA COPYRIGHT 2002 IFIS
ACCESSION NUMBER: 2001(06):B0823 FSTA
TITLE: Characterization of an oxygen-dependent inducible promoter, the nar promoter of Escherichia coli, to utilize in metabolic engineering.
AUTHOR: Se Jong Han; Ho Nam Chang; Jongwon Lee

CORPORATE SOURCE: Correspondence (Reprint) address, Jongwon Lee, Dep. of Biochem., Sch. of Med., Catholic Univ. of Taegu-Hyosung, Taegu 705-034, Korea. Tel. +82-53-650-4471. Fax +82-53-621-4106. E-mail leejw(a)cuth.cataegu.ac.kr
SOURCE: Biotechnology and Bioengineering, (2001) 72 (5) 573-576, 22 ref.
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The O.sub.2-dependent nar promoter from Escherichia coli, which can be induced by lowering the dissolved O.sub.2 (DO) concn. in the presence of nitrate ions, was studied to investigate its feasibility for use in metabolic engineering. Expression levels of the lacZ gene, encoding . **beta**.-**galactosidase**, under the control of the nar promoter were examined in E. coli grown initially under aerobic conditions (80% DO) to an OD.sub.6.sub.0.sub.0 of 35, followed by reduction of the DO level. Using the wild-type nar promoter in E.coli containing a mutation in the narG gene (encoding a subunit of nitrate reductase), specific . **beta**.-**galactosidase** activity could be maintained at constant levels (5000-20 000 **Miller units**) using various nitrate ion concn. Using a nitrate-independent nar promoter in a narL.sup.- E. coli mutant, specific .**beta**.-**galactosidase** activity could be maintained at a constant level of approx. 10 000 **Miller units** in the absence of nitrate ions. Results showed that the nar promoter system is a suitable expression system for metabolic engineering studies and for production of recombinant proteins in fed-batch culture.

L9 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5
ACCESSION NUMBER: 1998:301732 CAPLUS
DOCUMENT NUMBER: 129:77365
TITLE: Isolation and characterization of three Streptococcus pneumoniae transformation-specific loci by use of a lacZ reporter insertion vector
AUTHOR(S): Pestova, Ekaterina V.; Morrison, Donald A.
CORPORATE SOURCE: Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA
SOURCE: J. Bacteriol. (1998), 180(10), 2701-2710
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although more than a dozen new proteins are produced when Streptococcus pneumoniae cells become competent for genetic transformation, only a few of the corresponding genes have been identified to date. To find genes responsible for the prodn. of competence-specific proteins, a random lacZ transcriptional fusion library was constructed in S. pneumoniae by using the insertional lacZ reporter vector pEVP3. Screening the library for clones with competence-specific .**beta**.-**galactosidase** (.beta.-Gal) prodn. yielded three insertion mutants with induced .beta.-Gal levels of about 4, 10, and 40 **Miller units**. In all three clones, activation of the lacZ reporter correlated with competence and depended on competence-stimulating peptide. Chromosomal loci adjacent to the integrated vector were subcloned from the insertion mutants, and their nucleotide sequences were detd. Genes at two of the loci exhibited strong similarity to parts of Bacillus subtilis com operons. One locus contained open reading frames (ORFs) homologous to the comEA and comEC genes in B. subtilis but lacked a comEB homolog. A second locus contained four ORFs with homol. to the B. subtilis comG gene ORFs 1

to 4, but comG gene ORFs 5 to 7 were replaced in S. pneumoniae with an ORF
encoding a protein homologous to transport ATP-binding proteins. Genes at
all three loci were confirmed to be required for transformation by mutagenesis using pEVP3 for insertion duplications or an erm cassette for gene disruptions.

L9 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
ACCESSION NUMBER: 1998:65317 CAPLUS
DOCUMENT NUMBER: 128:214064
TITLE: Modulation of the function of the signal receptor domain of XylR, a member of a family of prokaryotic enhancer-like positive regulators
AUTHOR(S): Salto, Rafael; Delgado, Asuncion; Michan, Carmen; Marques, Silvia; Ramos, Juan L.
CORPORATE SOURCE: Department of Biochemistry, Molecular and Cellular Biology of Plants, Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Cientificas, Granada, E-18008, Spain
SOURCE: J. Bacteriol. (1998), 180(3), 600-604
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The XylR protein controls expression from the Pseudomonas putida TOL plasmid upper pathway operon promoter (Pu) in response to arom. effectors.
XylR-dependent stimulation of transcription from a Pu: lacZ fusion shows different induction kinetics with different effectors. With toluene, activation followed a hyperbolic curve with an apparent K of 0.95 mM and a
max. .**beta**.-**galactosidase** activity of 2,550
Miller units. With o-nitrotoluene, in contrast,
activation followed a sigmoidal curve with an apparent K of 0.55 mM and a Hill coeff. of 2.65. M-Nitrotoluene kept the XylR regulator in an inactive transcriptional form. Therefore, upon binding of an effector, the substituent on the arom. ring leads to productive or unproductive XylR
forms. The different transcriptional states of the XylR regulator are substantiated by XylR mutants. XylRE172K is a mutant regulator that is able to stimulate transcription from the Pu promoter in the presence of m-nitrotoluene; however, its response to m-aminotoluene was negligible, in
contrast with the wild-type regulator. These results illustrate the importance of the electrostatic interactions in effector recognition and in the stabilization of productive and unproductive forms by the regulator
upon arom. binding. XylRD135N and XylRD135Q are mutant regulators that are able to stimulate transcription from Pu in the absence of effectors, whereas substitution of Glu for Asp135 in XylRD135E resulted in a mutant whose ability to recognize effectors was severely impaired. Therefore, the conformation of mutant XylRD135Q as well as XylRD135N seemed to mimic that of the wild-type regulator when effector binding occurred, whereas mutant XylRD135E seemed to be blocked in a conformation similar to that of
wild-type XylR and XylRE172K upon binding to an inhibitor mol. such as m-nitrotoluene or m-aminotoluene.

L9 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
ACCESSION NUMBER: 1998:803860 CAPLUS
DOCUMENT NUMBER: 130:205625
TITLE: Development of a plasmid vector for overproduction of .**beta**.-**galactosidase** in Escherichia coli by using genetic components of groEx from symbiotic bacteria in Amoeba proteus

AUTHOR(S): Lee, Jung Eun; Ahn, Eun Young; Ahn, Tae In
CORPORATE SOURCE: Department of Biology Education, Seoul National University, Seoul, 151-742, S. Korea
SOURCE: J. Microbiol. Biotechnol. (1998), 8(5), 509-516
CODEN: JOMBES; ISSN: 1017-7825
PUBLISHER: Korean Society for Applied Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A plasmid vector, pXGPRMATG-lac-Tgx, was developed for overprodn. of .**beta.**-**galactosidase** in Escherichia coli using the genetic components of groEx, a heat-shock gene cloned from symbiotic X-bacteria in Amoeba proteus. The vector is composed of intragenic promoters P3 and P4 of groEx, the structural gene of lac operon, transcription terminator signals of lac and groEx, and ColE1 and amp' of pBluescript SKII. The optimized host, E. coli DH5.alpha., transformed with the vector constitutively produced 117,310-171,961 **Miller units** of .**beta.**-**galactosidase** per mg protein in crude ext. The amt. of enzyme in crude ext. was 53% of total water-sol. proteins. About 43% of the enzyme could be purified to a specific activity of 322,249 **Miller units**/mg protein after two-fold purifn., using two cycles of pptn. with ammonium sulfate and one step of gel filtration. Thus, the expression system developed in this study presents a low cost and simple method for purifying overproduced .**beta.**-**galactosidase** in E. coli.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 8
ACCESSION NUMBER: 1998:486413 CAPLUS
DOCUMENT NUMBER: 129:198611
TITLE: Fed-batch cultivation of an oxygen-dependent inducible promoter system, the nar promoter in escherichia coli with an inactivated nar operon
AUTHOR(S): Han, Se Jong; Chang, Ho Nam; Lee, Jongwon
CORPORATE SOURCE: Department of Chemical Engineering and Bioprocess Engineering Research Center, KAIST, Taejon, 305-701, S. Korea
SOURCE: Biotechnol. Bioeng. (1998), 59(4), 400-406
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nar promoter of Escherichia coli is maximally induced under anaerobic or microaerobic conditions in the presence of nitrate. We previously demonstrated in batch expts. that the intact nar promoter of E. coli cloned into a pBR322-based plasmid serves as a high-level expression system in a nar mutant of E. coli (J. Lee et al., 1996). In this study, we extend characterization of the nar promoter expression system to the fed-batch culture mode, which is widely used in industrial-scale fermn. From these expts., it was found that the specific .**beta.**-**galactosidase** activity expressed from the lacZ gene fused to the nar promoter was maximal when host cells were grown under aerobic conditions [dissolved oxygen, (DO) = 80%] to absorbance at 600 nm (OD600) = 35 before induction of the nar promoter by lowering DO to 1-2% with alternating microaerobic and aerobic conditions. Approx. 15 h after induction, the OD600 of the culture reached 135 and the specific .**beta.**-**galactosidase** activity increased to 40,000 **Miller units**, equiv. to approx. 35% of the total cellular proteins. The specific 3-galactosidase activity before induction was approx. ,000 **Miller units**, giving an induction ratio of approx. 40. Based on these results, we conclude that the nar promoter provides a convenient and effective high level expression system

under conditions of fed-batch culture.

L9 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 9
ACCESSION NUMBER: 1996:661923 CAPLUS
DOCUMENT NUMBER: 125:294710
TITLE: Characterization of an oxygen-dependent inducible promoter system, the nar promoter, and Escherichia coli with an inactivated nar operon
AUTHOR(S): Lee, Jintae; Cho, Moo Hwan; Lee, Jongwon
CORPORATE SOURCE: Dep. Chem. Eng., Yeungnam Univ., Kyungbuk, S. Korea
SOURCE: Biotechnol. Bioeng. (1996), 52(5), 572-578
CODEN: BIBIAU; ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nar promoter of Escherichia coli, which is maximally induced under anaerobic conditions in the presence of nitrate, was characterized to see whether the nar promoter cloned onto pBR322 can be used as an inducible promoter. To increase the expression level, the nar promoter was expressed in E. coli where active nitrate reductase cannot be expressed from the nar operon on the chromosome. A plasmid with the lacZ gene expressing **.beta.-galactosidase** instead of the structural genes of the nar operon was used to simplify an assay of induction of the nar promoter. The following effects were investigated to find optimal conditions: methods of inducing the nar promoter, optimal nitrate and molybdate concns. maximally inducing the nar promoter, the amt. of expressed **.beta.-galactosidase**, and induction ratio (specific **.beta.-galactosidase** activity after maximal induction/specific **.beta.-galactosidase** activity before induction.). The following results were obtained from the expts.: induction of the nar promoter was optimal when E. coli was grown in the presence of 1% nitrate at the beginning of culture; expression of **beta.-galactosidase** was not affected by molybdate; the induction ratio was maximal, approx. 300, when the overnight culture was grown in the flask for 2.5 h (OD 1.3) before being transferred to the fermentor; the amt. of **.beta.-galactosidase** per cell and per medium vol. was maximal when E. coli was grown under aerobic conditions to OD = 1.7; then the nar promoter was induced under microaerobic conditions made by lowering dissolved oxygen level (DO) to 1-2%. After approx. 6 h of induction, OD600 became 3.2 and specific . **beta.-galactosidase** activity became 36,000 **Miller units**, equiv. to 35% of total cellular proteins, which was confirmed from SDS-PAGE.

L9 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 10
ACCESSION NUMBER: 1996:112659 CAPLUS
DOCUMENT NUMBER: 124:167135
TITLE: Characterization of the nar promoter to use as an inducible promoter
AUTHOR(S): Lee, Jintae; Cho, Moohwan; Hong, Eock-Kee; Kim, Kwang-Soo; Lee, Jongwon
CORPORATE SOURCE: Dep. Chem. Eng., Yeungnam Univ., 705-034, S. Korea
SOURCE: Biotechnol. Lett. (1996), 18(2), 129-34
CODEN: BILED3; ISSN: 0141-5492
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nar promoter of Escherichia coli was characterized, which is maximally induced under anaerobic conditions in the presence of nitrate. The following results are obtained; Expression of **.beta.-galactosidase** was optimal at 1% of nitrate and was not affected much by molybdate; the amt. of **.beta.-galactosidase** per unit vol. was maximal when the nar promoter was induced at OD600 = 1.7, and when anaerobic condition was made by supplying nitrogen gas. At

the optimal condition, the ratio of .**beta**.-**galactosidase** between before and after induction was approx. 250 and **Miller units** were approx. 3500. The results showed that the nar promoter can be used as an inducible promoter.

L9 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 11
ACCESSION NUMBER: 1996:175149 CAPLUS
DOCUMENT NUMBER: 124:222089
TITLE: High-level expression of lacZ under control of the tac
or trp promoter using runaway replication vectors in Escherichia coli
AUTHOR(S): Kidwell, John; Kolibachuk; Dennis, Douglas
CORPORATE SOURCE: Dep. Biol., James Madison Univ., Harrisonburg, VA, 22807, USA
SOURCE: Biotechnol. Bioeng. (1996), 50(1), 108-14
CODEN: BIBIAU; ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To det. the utility of coupling runaway replication to the expression of cloned genes under the control of strong promoters, lacZ transcriptional fusions to the trp or tac promoter (Ptrp or Ptac) were constructed using plasmids in which the copy no. is thermally regulated. Cells contg. these plasmids were able to produce .**beta**.-**galactosidase** to levels between 3700 and 46,000 **Miller units** when induced only by a temp. upshift. The addn. of the appropriate chem. inducer, either IPTG (isopropyl-.beta.-D-thiogalactopyranoside) or IAA (3-.beta.-indoleacrylic acid), did not significantly enhance the thermal induction. The Ptac-controlled and Ptrp-controlled lacZ induction differed slightly in that the Ptac-controlled thermal induction exhibited a lag of approx. 1.5 h as compared to both chem. and thermal induction, whereas in the case of Ptrp-controlled induction, an increase in .**beta**.-**galactosidase** expression above background occurred at approx. the same time regardless of the means of induction. The best vector, a Ptrp-controlled lacZ fusion carried on a runaway replication vector having a basal copy no. of 10, was able to mediate the expression of .**beta**.-**galactosidase** to approx. 40,000 **Miller units** of .**beta**.-**galactosidase** comprising 25% of the total cell protein at 17 h postinduction under optimal conditions for protein yield. In these cells, lysis occurred as lacZ was maximally expressed. Under noninducing conditions, the plasmids were stable for at least 60 generations in the absence of antibiotic in batch culture.

L9 ANSWER 13 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:87706 BIOSIS
DOCUMENT NUMBER: PREV199799379419
TITLE: The construction and use of promoter probe vectors for Rhodococcus sp.
AUTHOR(S): Kayser, K. J.; Yun, C.-O.; Kilbane, J. J. Ii
CORPORATE SOURCE: Inst. Gas Technol., 1700 S. Mt. Prospect Rd., Des Plaines, IL 60018 USA
SOURCE: Actinomycetes, (1996) Vol. 7, No. 2, pp. 55-65.
ISSN: 0732-0574.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Three promoter probe vectors have been constructed for use within Rhodococcus sp. They are hybrid replicons capable of replicating both in E. coli and Rhodococcus species due to the presence of replication functions derived from pUC19 and the Rhodococcus fascians plasmid pRF29 respectively. Promoter probe vector pRCM1 contains a promoterless gene which encodes a membrane-associated chloramphenicol efflux protein (cmr) derived from Rhodococcus fascians plasmid pRF2, pRCAT3 contains a promoterless chloramphenicol acetyl transferase gene (cat) derived from Tn9, and pEBC26 contains a promoterless **beta**-

galactosidase gene derived from pSVB-gal. Many derivatives of pRCM1 and pRCAT3 receiving inserts that regulated the expression of chloramphenicol resistance in Rhodococcus sp. strain IGTS8 proved to be unstable in E. coli, frequently yielding plasmids containing deletions. This instability was found to be largely associated with these vectors; however, some inserts of Rhodococcus DNA increased and others alleviated this instability. Derivatives of pEBC26 were stable both in Rhodococcus and E. coli and many DNA fragments encoding Rhodococcus promoters were isolated. The size of these promoter-containing DNA fragments ranged from 0.15 to 3 Kb and the level of **beta-galactosidase** expression in Rhodococcus hosts ranged from 0.1 to 838 **Miller units**. Promoters from Rhodococcus were not observed to function in E. coli; however, the E. coli rrnB promoter was shown to function weakly in Rhodococcus.

L9 ANSWER 14 OF 19 FSTA COPYRIGHT 2002 IFIS
ACCESSION NUMBER: 1996(06):B0141 FSTA
TITLE: High-level expression of lacZ under control of the tac or trp promoter using runaway replication vectors in Escherichia coli.
AUTHOR: Kidwell, J.; Kolibachuk, D.; Dennis, D.
CORPORATE SOURCE: Correspondence (Reprint) address, D. Dennis, Dep. of Biol., James Madison Univ., Harrisonburg, VA 22807, USA
SOURCE: Biotechnology and Bioengineering, (1996) 50 (1) 108-114, 24 ref.
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Escherichia coli lacZ gene, encoding .**beta**.-**galactosidase**, was placed under control of the trp or tac promoter in the runaway replication vectors pRA95 and pRA96, in which copy number is thermally regulated. Expression of lacZ was examined in transformed cells containing these plasmids. Increasing the temp. increased expression of the lacZ gene; 41.degree.C was the optimum temp. for thermal induction of gene expression. Induction of gene expression using isopropyl-.beta.-D-thiogalactopyranoside (IPTG) or 3-.beta.-indoleacrylic acid IAA did not significantly enhance thermal induction of gene expression. In thermally induced strains harbouring the tac promoter, a lag period of approx. 1.5 h was observed prior to .**beta**.-**galactosidase** production; no apparent lag was observed in strains possessing the trp promoter. Max. .**beta**.-**galactosidase** levels (up to 46 000 **Miller units**) were produced using a trp promoter on pRA96, having a basal copy number of 10; enzyme levels were 25% of the total cell protein 17 h after thermal induction.

L9 ANSWER 15 OF 19 FSTA COPYRIGHT 2002 IFIS
ACCESSION NUMBER: 1997(02):B0111 FSTA
TITLE: Characterization of an oxygen-dependent inducible promoter system, the nar promoter, and Escherichia coli with an inactivated nar operon.
AUTHOR: Jintae Lee; Moo Hwan Cho; Jongwon Lee
CORPORATE SOURCE: Correspondence (Reprint) address, Jongwon Lee, Dep. of Biochem., Sch. of Med., Catholic Univ. of Taegu-Hyosung, 3056-6, Daemyung 4-Dong, Nam-Gu, Taegu 705-034, Korea. Tel. 82-53-650-4471. Fax 82-53-621-4106
SOURCE: Biotechnology and Bioengineering, (1996) 52 (5) 572-578, 22 ref.
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nar promoter of Escherichia coli, which is optimally induced in the

presence of nitrate under anaerobic conditions, was characterized in order to ascertain its usefulness as an inducible promoter. The nar promoter was expressed in an E. coli strain having a mutant nar operon which does not express active nitrate reductase. A plasmid containing the lacZ gene, expressing .**beta**.-**galactosidase**, instead of the structural genes of the nar operon was used to assay induction of the nar promoter. Optimal conditions for nar induction were analysed. Results showed that induction of the nar promoter was optimal when E. coli was grown initially in the presence of 1% nitrate. Expression of the lacZ gene was not affected by molybdate ions. The amount of .**beta**.-**galactosidase** per cell and per medium vol. was max. when E. coli was grown under aerobic conditions to an optical density (at 600 nm) of 1.7; induction of the nar promoter was observed by lowering dissolved O.sub.2 concn. to microanaerobic levels (1-2%). After approx. 6 h induction, specific .**beta**.-**galactosidase** activity was 36 000 **Miller units**, equivalent to 35% of total cellular proteins, which was confirmed by SDS-PAGE. The specific activity of .**beta**.-**galactosidase** expressed from the nar promoter was comparable to that obtained using the tac and VHb promoters.

L9 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 12
ACCESSION NUMBER: 1993:54760 CAPLUS
DOCUMENT NUMBER: 118:54760
TITLE: Experimental conditions may affect reproducibility of the .**beta**.-**galactosidase** assay
AUTHOR(S): Giacomini, Alessio; Corich, Viviana; Ollero, Francisco J.; Squartini, Andrea; Nuti, Marco P.
CORPORATE SOURCE: Dip. di Biotecnol. Agrarie, Univ. Stud. Padova, Padoua, Italy
SOURCE: FEMS Microbiol. Lett. (1992), 100(1-3), 87-90
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Several exptl. conditions and parameters contributing to the detn. of .**beta**.-**galactosidase** activity, as proposed in the J. H. Miller (1972) assay, were studied. This assay is based on bacterial cell permeabilization followed by spectrophotometric measurement of o-nitrophenol released from its galactoside conjugate. Use of the absorbance correction factor and the nature and concn. of permeabilizing agents were taken into account as different exptl. conditions. Reaction time, culture vol., and growth stage were investigated as equation parameters. From a quant. point of view the results, in terms of **Miller units**, are markedly affected by variation in these conditions. Therefore, to ensure reproducibility it is advisable to use const. values for all the parameters.

L9 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:546672 CAPLUS
DOCUMENT NUMBER: 113:146672
TITLE: Low copy number plasmids for regulated low-level expression of cloned genes in Escherichia coli with blue/white insert screening capability
AUTHOR(S): Lerner, Claude G.; Inouye, Masayori
CORPORATE SOURCE: Robert Wood Johnson Med. Sch., Rutgers, State Univ., Piscataway, NJ, 08854-5635, USA
SOURCE: Nucleic Acids Res. (1990), 18(15), 4631
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors constructed pCL1920 and pCL1921, a pair of low-copy-no. plasmids which contain a 580-bp BstUI fragment that carries the lac

promoter/operator, multiple cloning sites, and lacZ fragment of pUC19 cloned in place of the polylinker region in pGB2, a pSC101-derived plasmid which confers spectinomycin (50 .mu.g/mL) and streptomycin (100 .mu.g/mL) resistance in E. coli. All multiple cloning sites indicated are unique except for an addnl. EcoRI site. Plasmids pCL19020 and pCL1921 contain the BstUI fragment in opposite orientations with respect to the pGB2 sequences. In the absence of inducer the pCL1920/21 vectors do not produce detectable levels of .**beta**.-**galactosidase** in JM105 (lacq lacZ.DELTA.M15) cells (<2 **Miller units**). In the presence of 2 mM IPTG (isopropyl-.beta.-D-thiogalactopyranoside) the .**beta**.-**galactosidase** levels of the pCL1920/21 [JM105] transformants rose to 11 units, whereas the pUC19 [JM105] transformants produced 470 units; a 43-fold increase. These results are consistent with the expected 40-fold difference in plasmid copy no. between pCL1920/21 (5 copies/cell) compared to that of thepUC vectors (200 copies/cell). Thus, the pCL 1920 and pCL1921 vectors allow regulated low-level expression of genes inserted downstream of the lac promoter-operator when transformed into strains contg. the lacq gene. They should also be useful for cloning genes which may be deleterious at high copy no. Since the pCL1020/21 vectors are compatible with ColE-derived plasmids, they can be used to form stable co-transformants together with pBR322 or pUC-derived plasmids. For blue/white screening of inserts, competent host cells with the lacZ.DELTA.M15 gene are used, and the transformation mixt. is plated on LB, spectinomycin plates pre-spread with 5 .mu.L of 0.2 M IPTG and 25 .mu.L 40 mg/mL 5-bromo-4-chloro-indolyl-.beta.-D-galactopoyranoside per plate.

L9 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 13
ACCESSION NUMBER: 1988:568937 CAPLUS
DOCUMENT NUMBER: 109:168937
TITLE: Fermentation of lactose by Zymomonas mobilis carrying a Lac+ recombinant plasmid
AUTHOR(S): Yanase, Hideshi; Kurii, Junn; Tonomura, Kenzo
CORPORATE SOURCE: Coll. Agric., Univ. Osaka Prefect., Osaka, 591, Japan
SOURCE: J. Ferment. Technol. (1988), 66(4), 409-15
CODEN: JFTED8; ISSN: 0385-6380
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lac+ recombinant plasmids encoding a .**beta**.-**galactosidase** fused protein and lactose permease of Escherichia coli were introduced into Z. mobilis. The fused protein was expressed with 450 to 5860 **Miller units** of .**beta**.-**galactosidase** activity, and functioned as lactase. Raffinose uptake by Z. mobilis CP4 was enhanced in the plasmid-carrying strain over the plasmid-free strain, suggesting that the lactose permease was functioning in the organism. Z. mobilis Carrying the plasmid could produce EtOH from lactose and whey, but could not grown on lactose as sole C source. Its growth was inhibited by either galactose or the galactose liberated from lactose.

L9 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 14
ACCESSION NUMBER: 1986:201248 CAPLUS
DOCUMENT NUMBER: 104:201248
TITLE: Construction of a new vector for the expression of foreign genes in Zymomonas mobilis
AUTHOR(S): Byun, M. O. K.; Kaper, J. B.; Ingram, L. O.
CORPORATE SOURCE: Dep. Microbiol. Cell Sci., Univ. Florida, Gainesville, FL, 32611, USA
SOURCE: J. Ind. Microbiol. (1986), 1(1), 9-15
CODEN: JIMIE7

DOCUMENT TYPE: Journal
LANGUAGE: English

AB Broad host range plasmids are suitable as vectors to introduce antibiotic resistance genes into Z. mobilis. However, attempts to use these vectors to carry other genes with enteric promoters and controlling elements have resulted in limited success due to poor expression. Thus, a promoter cloning vector was constructed in a modified pBR327 and used this vector to isolate 12 promoters from Z. mobilis which express various levels of .**beta**.-**galactosidase** in Escherichia coli. Four of these were then subcloned into pCVD 305 for introduction into Z. mobilis. All expressed .**beta**.-**galactosidase** in Z. mobilis with activities of 100-1800 **Miller units**. One of these retained a BamHI site into which new genes can be readily inserted immediately downstream from the Z. mobilis promoter. Genetic traits carried by pCVD 305 were initially unstable but spontaneous variants were produced during subculture in which the plasmid was resistant to curing at elevated temp. One of these variants was examd. The increased stability of this variant results from an alteration in the plasmid rather than a chromosomal mutation or from chromosomal integration.

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     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
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               SEA LACTOCOCCUS(W)LACTIS
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                 3   FILE ADISALERTS
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               526   FILE BIOBUSINESS
                12   FILE BIOCOMMERCE
              2620   FILE BIOSIS
               663   FILE BIOTECHABS
               663   FILE BIOTECHDS
              1535   FILE BIOTECHNO
              4137   FILE CABA
                40   FILE CANCERLIT
              2854   FILE CAPLUS
               321   FILE CEABA-VTB
                 1   FILE CEN
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                41   FILE CONFSCI
                12   FILE DDFU
              1111   FILE DGENE
                 2   FILE DRUGNL
                15   FILE DRUGU
                 2   FILE DRUGUPDATES
                16   FILE EMBAL
              1463   FILE EMBASE
              1157   FILE ESBIOBASE
                 1   FILE FOREGE
              1004   FILE FROSTI
              1691   FILE FSTA
              1211   FILE GENBANK
                 4   FILE HEALSAFE
                72   FILE IFIPAT
               262   FILE JICST-EPLUS
                19   FILE KOSMET
              1563   FILE LIFESCI
              1839   FILE MEDLINE
                 1   FILE NIOSHTIC
                 3   FILE NTIS
              1746   FILE PASCAL
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                 4   FILE PHIN
                39   FILE PROMT
              2800   FILE SCISEARCH
               630   FILE TOXCENTER
               768   FILE TOXLIT
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              299   FILE USPATFULL
                1   FILE USPAT2
              159   FILE WPIDS
              159   FILE WPINDEX
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     FILE 'CABA, CAPLUS, SCISEARCH, BIOSIS' ENTERED AT 13:07:27 ON 13 MAR 2002
L2            84 S L1 AND PERMEAB?
L3            45 DUP REM L2 (39 DUPLICATES REMOVED)
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L3 ANSWER 36 OF 45 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 17
ACCESSION NUMBER: 1992:586759 CAPLUS
DOCUMENT NUMBER: 117:186759
TITLE: Effect of the unsaturation of phospholipid acyl chains on leucine transport of **Lactococcus lactis** and membrane **permeability**
AUTHOR(S): In 't Veld, Gerda; Driessen, Arnold J. M.; Konings, Wil N.
CORPORATE SOURCE: Dep. Microbiol., Univ. Groningen, Haren, 9751 NN, Neth.
SOURCE: Biochim. Biophys. Acta (1992), 1108(1), 31-9
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of the degree of unsatn. of the phospholipid acyl chains on the branched-chain amino acid transport system of L. lactis was investigated by the use of a membrane fusion technique. Transport activity was analyzed in hybrid membranes composed of equimolar mixts. of synthetic unsatd. phosphatidylenthanolamine (PE) and phosphatidylcholine (PC) in which the no. of cis double bonds in the 18-carbon acyl chains was varied.
The accumulation level and initial rate of both counterflow and protonmotive-force driven transport of leucine decreased with increasing no. of double bonds. The redn. in transport activity with increasing no. of double bonds correlated with an increase in the passive **permeability** of the membranes to leucine. The membrane fluidity was hardly affected by the double bond content. It is concluded that the degree of lipid acyl chain unsatn. is a minor determinant of the activity of the branched chain amino acid transport system, but effects strongly the passive **permeability** of the membrane.

L3 ANSWER 37 OF 45 CABA COPYRIGHT 2002 CABI DUPLICATE 18
ACCESSION NUMBER: 92:1627 CABA
DOCUMENT NUMBER: 920449928
TITLE: The bacteriocin lactococcin A specifically increases **permeability** of lactococcal cytoplasmic membranes in a voltage-independent, protein-mediated manner
AUTHOR: Belkum, M. J. van; Kok, J.; Venema, G.; Holo, H.; Nes, I. F.; Konings, W. N.; Abee, T.; Van Belkum, M. J.
CORPORATE SOURCE: Department of Genetics, University of Groningen, Kerklaan 30, 9751NN Haren, Netherlands.
SOURCE: Journal of Bacteriology, (1991) Vol. 173, No. 24, pp. 7934-7941. 36 ref.
ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lactococcin A is a bacteriocin produced by **Lactococcus lactis**. Its structural gene has recently been cloned and sequenced. Purified lactococcin A increased the **permeability** of the cytoplasmic membrane of L. lactis and dissipated the membrane potential. A significantly higher concentration of lactococcin A was needed to dissipate the membrane potential in an immune strain of L.

lactis. Lactococcin A at low concentrations (0.029 micro g/mg of protein) inhibited secondary and phosphate-bond driven transport of amino acids in sensitive cells and caused efflux of preaccumulated amino acids. Accumulation of amino acids by immune cells was not affected by this concentration of lactococcin A. Lactococcin A also inhibited proton motive force-driven leucine uptake and leucine counterflow in membrane vesicles of the sensitive strain but not in membrane vesicles of the immune strain. These observations indicate that lactococcin A makes the membrane **permeable** for leucine in the presence or absence of a proton motive force and that the immunity factor(s) is membrane linked. Membrane vesicles of Clostridium acetobutylicum, Bacillus subtilis and Escherichia coli were not affected by lactococcin A, nor were liposomes derived from phospholipids of L. lactis. These results indicate that lactococcin A acts on the cytoplasmic membrane and is very specific towards lactococci. The combined results obtained with cells, vesicles and liposomes suggest that the specificity of lactococcin A may be mediated by a receptor protein associated with cytoplasmic membrane.

L3 ANSWER 38 OF 45 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 91:688106 SCISEARCH
THE GENUINE ARTICLE: GV187
TITLE: THE BACTERIOCIN LACTOCOCCIN-A SPECIFICALLY INCREASES **PERMEABILITY** OF LACTOCOCCAL CYTOPLASMIC MEMBRANES IN A VOLTAGE-INDEPENDENT, PROTEIN-MEDIATED MANNER
AUTHOR: VANBELKUM M J (Reprint); KOK J; VENEMA G; HOLO H; NES I F; KONINGS W N; ABEE T
CORPORATE SOURCE: UNIV GRONINGEN, DEPT GENET, KERKLAAN 80, 9751 NN HAREN, NETHERLANDS (Reprint); NLVF, MICROBIAL GENE TECHNOL LAB, N-1432 AS, NORWAY; UNIV GRONINGEN, DEPT MICROBIOL, 9751 NN HAREN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS; NORWAY
SOURCE: JOURNAL OF BACTERIOLOGY, (1991) Vol. 173, No. 24, pp. 7934-7941.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Lactococcin A is a bacteriocin produced by **Lactococcus lactis**. Its structural gene has recently been cloned and sequenced (M. J. van Belkum. B. J. Hayema, R. E. Jeeninga, J. Kok, and G. Venema, Appl. Environ. Microbiol. 57:492-498, 1991). Purified lactococcin A increased the **permeability** of the cytoplasmic membrane of L. lactis and dissipated the membrane potential. A significantly higher concentration of lactococcin A was needed to dissipate the membrane potential in an immune strain of L. lactis. Lactococcin A at low concentrations (0.029-mu-g/mg of protein) inhibited secondary and phosphate-bond driven transport of amino acids in sensitive cells and caused efflux of preaccumulated amino acids. Accumulation of amino acids by immune cells was not affected by this concentration of lactococcin A. Lactococcin A also inhibited proton motive force-driven leucine uptake and leucine counterflow in membrane vesicles of the sensitive strain but not in membrane vesicles of the immune strain. These observations indicate that lactococcin A makes the membrane **permeable** for leucine in the presence or absence of a proton motive force and that the immunity factor(s) is membrane linked. Membrane vesicles of Clostridium acetobutylicum, Bacillus subtilis, and Escherichia coli were not affected by lactococcin A, nor were liposomes derived from phospholipids of L. lactis. These results indicate that lactococcin A acts on the cytoplasmic membrane and is verv specific towards lactococci. The combined results

obtained with cells, vesicles, and liposomes suggest that the specificity of lactococcin A be mediated by a receptor protein associated with the cytoplasmic membrane.

L3 ANSWER 39 OF 45 CABA COPYRIGHT 2002 CABI DUPLICATE 19
ACCESSION NUMBER: 91:11466 CABA
DOCUMENT NUMBER: 910443783
TITLE: Characterization of lactococci and lactobacilli isolated from semihard goats' cheese
AUTHOR: Requena, T.; Pelaez, C.; Desmazeaud, M. J.
CORPORATE SOURCE: Instituto del Frio (CSIC), Ciudad Universitaria, 28040 Madrid, Spain.
SOURCE: Journal of Dairy Research, (1991) Vol. 58, No. 1, pp. 137-145. 32 ref.
ISSN: 0022-0299
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Several strains of **Lactococcus lactis** var. lactis, Lactobacillus casei and Lactobacillus plantarum isolated from traditional goat milk cheese were studied for titratable acidity, proteolysis in milk and enzymic activities. Aminopeptidase activities were measured with whole cells and cells made **permeable** with Triton X-100. Caseinolytic activity was investigated using PAGE. Lc. lactis var. lactis had a level of proteolytic activity in skim milk greater than that of Lb. casei, while this activity in Lb. plantarum was very low. Alanine aminopeptidase activity was almost non-existent for all strains tested, while lysine aminopeptidase activity appeared to be of fundamentally intracellular origin. Leucine aminopeptidase activity was also greater in **permeable** cells than in whole cells for Lb. casei and Lb. plantarum. Lc. lactis var. lactis leucine aminopeptidase activity was greater in whole cells. No significant hydrolysis of casein was found with Lb. casei IFPL 725 and Lb. plantarum IFPL 722 made **permeable** with Triton X-100 after 24 h incubation with whole bovine casein.

L3 ANSWER 40 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 89:65434 CABA
DOCUMENT NUMBER: 890432541
TITLE: Characterization of an aminopeptidase of Streptococcus cremoris AM2 and an alpha -galactosidase of Leuconostoc lactis CNRZ 1091
Caracterisation d'une aminopeptidase chez Streptococcus cremoris AM2 et d'une alpha -galactosidase chez Leuconostoc lactis CNRZ 1091
AUTHOR: Boquien, C. Y.; Desmazeaud, M. J.; Corrieu, G.
CORPORATE SOURCE: INRA, Lab. Genie des Procedes Biotech. Agro-alimentaires, 78850 Thiverval-Grignon, France.
SOURCE: Lait, (1989) Vol. 69, No. 1, pp. 71-81. 21 ref.
DOCUMENT TYPE: Journal
LANGUAGE: French
SUMMARY LANGUAGE: English
AB The API ZYM enzyme system was used to detect enzymic activities specific to lactic acid bacteria. A total of 20 mesophilic streptococcal and 34 leuconostoc strains were tested, and 2 enzymes selected for characterization were (i) an aminopeptidase of Streptococcus cremoris AM2 that hydrolysed histidyl-phenylalanine- beta -naphthylamide and (ii) an alpha -galactosidase of Leuconostoc lactis CNRZ 1091 that hydrolysed paranitrophenol- alpha -galactose. Activity of (i) was max. in fresh cells whilst that of (ii) was max. when the cells were **permeabilized** with Triton X-100. Enzymes (i) and (ii) resp. had pH optimum of 7 and 6.5, and optimum temp. was 40 deg C for both enzymes. Apparent Michaelis

constant (Km) was 0.17 and 0.73 mM for (i) and (ii) resp., and Vmax was 0.6 and 90 pmol/s per 107 c.f.u. per ml.

L3 ANSWER 41 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 84:85357 CABA
DOCUMENT NUMBER: 840491300
TITLE: Fermentation process improvement by membrane technology
AUTHOR: Kyung, K. H.
CORPORATE SOURCE: Michigan State Univ., East Lansing, Michigan 48824, USA.
SOURCE: Dissertation Abstracts International, B (Sciences and Engineering), (1984) Vol. 44, No. 7, pp. 2071-2072.
Order No: DA8324736.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB For continuous fermentation of glucose to ethanol using Saccharomyces cerevisiae ATCC 4126, substrate was fed into a continuous dialysate circuit, then transferred by membrane diffusion through an intermediate dialyser into a batch fermentor circuit; product was simultaneously withdrawn from the fermentor circuit through dialyser membranes into the dialysate circuit and out in the effluent. Cells in the fermentor were essentially immobilized, and converted substrate to product by maintenance metabolism. Advantages of the system were offset by low productivity which was apparently limited by membrane **permeability**. A batch fermentation system utilizing a mutualistic dialysis culture of Streptococcus lactis and Candida utilis was also investigated, the 2 organisms being inoculated into separate fermentors connected by an intermediate dialyser. Lactose was fermented by S. lactis to lactic acid which was dialysed into the C. utilis culture and utilized to produce yeast biomass. Solute exchange rate across the membrane was the primary limiting factor.

L3 ANSWER 42 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 84:76732 CABA
DOCUMENT NUMBER: 840491084
TITLE: Location of peptidases outside and inside the membrane of Streptococcus cremoris
AUTHOR: Exterkate, F. A.
CORPORATE SOURCE: Netherlands Inst. for Dairy Res., NIZO, Ede, Netherlands.
SOURCE: Applied and Environmental Microbiology, (1984) Vol. 47, No. 1, pp. 177-183. 24 ref.
ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Peptidase activity determinations involving native cells of Streptococcus cremoris and completely disrupted cell preparations, as well as experiments concerned with peptidase activity distribution among cell fractions obtained by a damage-restrictive removal of the cell wall and release of intracellular material, suggest the presence of peptidases with distinguishable locations. Alanyl, leucyl and prolyl aminopeptidase activities are most likely located in the cell wall-membrane interface, showing no detectable association with the membrane. Lysyl aminopeptidase is present not only in this location, but also as an intracellular enzyme. Endopeptidase activity and glutamate aminopeptidase activity appear to be weakly associated with the membrane. Results of experiments concerned with **permeabilization** of the membrane, and findings regarding an effect of the local environment of the enzymes on their pH activity profiles are evaluated and considered as being indicative of the proposed location. The

possible implications of these findings with respect to protein utilization during growth of the organism in milk discussed.

L3 ANSWER 43 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 82:16001 CABA
DOCUMENT NUMBER: 810469483
TITLE: Regulation of methyl- beta -D-thiogalactopyranoside-6-phosphate accumulation in Streptococcus lactis by exclusion and expulsion mechanisms
AUTHOR: Thompson, J.; Saier, M. H., Jr.
CORPORATE SOURCE: Dep. of Biol., John Muir Coll., Univ. of California at San Diego, La Jolla, California 92093, USA.
SOURCE: Journal of Bacteriology, (1981) Vol. 146, No. 3, pp. 885-894. 40 ref.
ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Starved cells of Streptococcus lactis ML3 (grown previously on galactose, lactose or maltose) accumulated methyl- beta -D-thiogalactopyranoside (TMG) by the lactose:phosphotransferase system. >98% of accumulated sugar was present as a phosphorylated derivative, TMG-6-phosphate (TMG-6P). When a phosphotransferase system sugar (glucose, mannose, 2-deoxyglucose, or lactose) was added to the medium simultaneously with TMG, the beta -galactoside was excluded from the cells. Galactose enhanced the accumulation of TMG-6P. Glucose, mannose, lactose, or maltose + arginine, when added to a suspension of TMG-6P-loaded cells of S. lactis ML3, elicited rapid expulsion of intracellular solute. The material recovered in the medium was exclusively free TMG. Expulsion of galactoside required both entry and metabolism of an appropriate sugar, and intracellular dephosphorylation of TMG-6P preceded efflux of TMG. The rate of dephosphorylation of TMG-6P by **permeabilized** cells was increased 2-3X by adenosine 5'-triphosphate but was strongly inhibited by fluoride. S. lactis ML3 (DGr) was derived from S. lactis ML3 by positive selection for resistance to 2-deoxy-D-glucose and was defective in the enzyme IIMan component of the glucose:phosphotransferase system. Neither glucose nor mannose excluded TMG from cells of S. lactis ML3 (DGr), and these 2 sugars failed to elicit TMG expulsion from preloaded cells of the mutant strain. Accumulation of TMG-6P by S. lactis ML3 can be regulated by two independent mechanisms whose activities promote exclusion or expulsion of galactoside from the cell.

L3 ANSWER 44 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 82:19154 CABA
DOCUMENT NUMBER: 820474332
TITLE: Membrane-bound peptidases in Streptococcus cremoris
AUTHOR: Exterkate, F. A.; International Dairy Federation, Symposium
CORPORATE SOURCE: Netherlands Inst. for Dairy Res. (NIZO), Ede, Netherlands.
SOURCE: Netherlands Milk and Dairy Journal, (1981) Vol. 35, No. 3/4, pp. 328-332. 12 ref.
ISSN: 0028-209X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Review of results of various experiments, relating to peptidase activity distribution among cell fractions, **permeabilization** of the membrane, and initial reaction kinetics, leads to the conclusion that Streptococcus cremoris has a functional system of specific peptidases located within the membrane and at its outside surface. This system plays a role in protein utilization during growth of the organism in milk, by making small peptides and free amino acids available for transport through

the membrane.

L3 ANSWER 45 OF 45 CABA COPYRIGHT 2002 CABI
ACCESSION NUMBER: 80:20598 CABA
DOCUMENT NUMBER: 800458595
TITLE: Effect of external factors on manifestation of antagonistic activity by lactic streptococci
AUTHOR: Zaborskikh, E. I.
SOURCE: Biologiya mikroorganizmov i ikh ispol'zovanie v narodnom khozyaistve, (1977) pp. 99-103, 208. 10 ref.
Publisher: Gosudarstvennyi Universitet imeni A. A. Zhdanova. Irkutsk
PUB. COUNTRY: USSR
DOCUMENT TYPE: Miscellaneous
LANGUAGE: Russian

AB Cultures of 2 strains of Streptococcus lactis and 2 of Str. diacetilactis showing antagonism against Escherichia coli were filtered through membrane filters; the filtrates and hydrolysed milk as control were inoculated with E. coli at 20 to 2 X 108 cells/ml, and growth intensity measured by nephelometer after incubation for 24 h at 30 deg C. The filtrates inhibited E. coli growth irrespective of size of inoculum. The antimicrobial compounds formed by the strains studied were stable to heating for 30 min at 65 deg C or to boiling for 20 min; they retained their activities in acid, but were inactivated under alkaline conditions; they passed through semi-**permeable** membranes; they were not precipitated by trichloroacetic acid, saturated (NH4)2SO4 solution, ammonia or ethanol; and they were not extractable by diethyl ether.